



## **Impact of inhalation anaesthesia, surgery and analgesic treatment on home cage behaviour in laboratory mice**

Cesarovic, Nikola ; Arras, Margarete ; Jirkof, Paulin

**Abstract:** Anaesthesia and analgesia are used frequently in laboratory routine to ensure animal welfare and good scientific outcomes in experiments that may elicit pain or require immobilisation of the animal. However, there is concern regarding the effect of these procedures on animal behaviour in subsequent experiments. Our study determined the impact of short inhalation anaesthesia (sevoflurane, 15 min, 4.9%) and minor surgery (one-sided sham embryo transfer in females, one-sided sham vasectomy in males) with or without pain treatment (carprofen, 5 mg/kg, bid) on spontaneous species-specific home cage behaviours in inbred mice. Analysis of 18-h continuous video recordings showed clear post-procedural changes in spontaneous home cage behaviours, with changes of a moderate level after anaesthesia being marked after surgery. Self-grooming, resting and locomotion were the most important behaviours for group separation. Analysis of the temporal distribution of behavioural changes revealed that resting behaviour was altered contradictory to its circadian rhythm as it was decreased in the light phase and increased in the dark phase. Also, locomotion was decreased in the dark phase at 12 to 18 h after surgery and anaesthesia. In contrast, self-grooming was increased independently of circadian rhythm, being increased for up to 18 h after surgery and anaesthesia. Following surgery, there was no significant difference in duration of behaviours between animals that were treated with carprofen or left without pain relief. In conclusion, it can be assumed that the changes observed in home cage behaviours hint at reduced animal well-being. However, pain or the efficacy of post-operative pain treatment could not be discriminated reliably from the impact of the surgical procedure including inhalation anaesthesia by observing animals' home cage behaviour. However for the interpretation of behavioural research data, the distinct impact of anaesthesia, surgery, pain treatment and other experimental procedures has to be considered. Our results highlight the requirement for knowledge of species-specific circadian rhythms of behaviours as well as the importance of determining the appropriate time of day for behavioural and welfare assessment.

DOI: <https://doi.org/10.1016/j.applanim.2014.04.010>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-97554>

Journal Article

Accepted Version

Originally published at:

Cesarovic, Nikola; Arras, Margarete; Jirkof, Paulin (2014). Impact of inhalation anaesthesia, surgery and analgesic treatment on home cage behaviour in laboratory mice. *Applied Animal Behaviour Science*, 157:137-145.

DOI: <https://doi.org/10.1016/j.applanim.2014.04.010>

## Accepted Manuscript

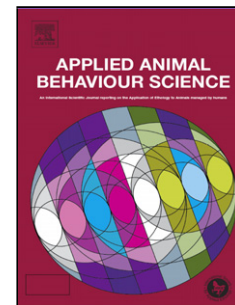
Title: Impact of inhalation anaesthesia, surgery and analgesic treatment on home cage behaviour in laboratory mice

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PII: S0168-1591(14)00113-0  
DOI: <http://dx.doi.org/doi:10.1016/j.applanim.2014.04.010>  
Reference: APPLAN 3896

To appear in: *APPLAN*

Received date: 10-12-2013  
Revised date: 14-4-2014  
Accepted date: 22-4-2014



Please cite this article as: Cesarovic, N., Arras, M., Jirkof, P., Impact of inhalation anaesthesia, surgery and analgesic treatment on home cage behaviour in laboratory mice, *Applied Animal Behaviour Science* (2014), <http://dx.doi.org/10.1016/j.applanim.2014.04.010>

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**Impact of inhalation anaesthesia, surgery and analgesic  
treatment on home cage behaviour in laboratory mice**

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**Key words:** mice, anaesthesia, surgery, behaviour, refinement, animal well-being

## Abstract

Anaesthesia and analgesia are used frequently in laboratory routine to ensure animal welfare and good scientific outcomes in experiments that may elicit pain or require immobilisation of the animal. However, there is concern regarding the effect of these procedures on animal behaviour in subsequent experiments. Our study determined the impact of short inhalation anaesthesia (sevoflurane, 15 min, 4.9%) and minor surgery (one-sided sham embryo transfer in females, one-sided sham vasectomy in males) with or without pain treatment (carprofen, 5 mg/kg, bid) on spontaneous species-specific home cage behaviours in inbred mice. Analysis of 18-hour continuous video recordings showed clear post-procedural changes in spontaneous home cage behaviours, with changes of a moderate level after anaesthesia being marked after surgery. Self-grooming, resting and locomotion were the most important behaviours for group separation. Analysis of the temporal distribution of behavioural changes revealed that resting behaviour was altered contradictory to its circadian rhythm as it was decreased in the light phase and increased in the dark phase. Also, locomotion was decreased in the dark phase at 12 to 18 hours after surgery and anaesthesia. In contrast, self-grooming was increased independently of circadian rhythm, being increased for up to 18 hours after surgery and anaesthesia. Following surgery, there was no significant difference in duration of behaviours between animals that were treated with carprofen or left without pain relief. In conclusion, it can be assumed that the changes observed in home cage behaviours hint at reduced animal well-being. However, pain or the efficacy of post-operative pain treatment could not be discriminated reliably from the impact of the surgical procedure including inhalation anaesthesia by observing animals' home cage behaviour. However for the interpretation of behavioural research data, the distinct impact of anaesthesia, surgery, pain treatment and other experimental procedures has to be considered. Our results highlight the requirement for knowledge of species-specific circadian rhythms of behaviours as well as the importance of determining the appropriate time of day for behavioural and welfare assessment.

## 1 Introduction

Laboratory mice are currently the most widely used mammal species in biomedical research (Baumanns, 2004). Due to their manageable size, a wealth of inbred or genetically modified strains and plenitude of established experimental protocols, mice are used increasingly in complex investigations. These often require induction of general anaesthesia for performing special diagnostic manipulations (e.g., imaging procedures, endoscopy, blood collection), or surgical procedures that in turn require peri- and/or post-operative pain treatment. Analgesic treatment would seem necessary after invasive procedures like laparotomy, but has been omitted frequently in the past (Richardson and Flecknell, 2006; Stokes et al., 2009). Reasons may vary from concern that analgesic use may compromise the data obtained from the proven model to the difficulties of detecting and interpreting signs of pain after minor surgery in mice (Richardson and Flecknell, 2006).

In contrast to most physiological and clinical parameters, behaviour can be recorded easily in a non-invasive manner and can provide a sensitive correlate of the internal state of an animal. Alterations in the frequency of, or in the latency to display, spontaneous and species-specific behaviours, like rearing, sniffing, walking or burrowing behaviour (Roughan et al., 2009; Jirkof et al., 2010), as well as the quality of nest construction and structuring of cage territory (Arras et al., 2007; Jirkof et al., 2013b) are recent examples of behavioural indicators of well-being or distress and also pain in mice. Thus, behavioral indicators are frequently used not only in the clinical assessment of laboratory animal well-being but also in basic pain research.

Recently, it has become apparent that the physiological and behavioural changes induced by minor to moderate surgery can last up to 24 - 48 hours (Arras et al., 2007; Matsumiya et al., 2012). Moreover, it has been shown that changes induced by anaesthesia, and possibly also by treatment-related procedures (e.g. handling, transport to operating theatre etc.), may affect physiology and animal well-being for several hours (Cesarovic et al., 2010; Jirkof et al., 2013b). It can be assumed that, in some situations, the effects of anaesthesia may overlap and to some extent mask the post-operative effects of pain and/or analgesic treatment. In addition, although the impact of volatile anaesthetic agents on learning, memory, solving of

spatial tasks and activity has been studied recently (Petrenko et al., 2008; Valentim et al., 2008; Mena et al., 2010), the effects of anaesthesia, as an integral part of standard surgical procedures, on spontaneous home cage behaviours have been described only rarely. Thus this study aimed to deepen the knowledge on mouse behaviour in the period following short anaesthesia or minor surgical procedure. Further, there is concern regarding not only animal welfare but also the reliability of data obtained from research using animals that have undergone procedures that may elicit pain and/or involve analgesic and/or anaesthetic treatment. In some areas of biomedical research like in sepsis or acute brain injury models experimental read-out is recorded shortly post-procedural (Baracchi et al., 2011; Khatibi et al., 2011; Kuroki et al., 2013). For such procedures inhalation anaesthesia seems to be the protocol of choice. Hence, questions regarding the duration and persistence of long-lasting anaesthetic or procedural effects are rising into focus not only in behavioural welfare assessment but also basic and preclinical research (Stokes et al., 2009). To our knowledge there is only one publication presenting changes in home cage behaviours after surgery and anaesthesia over a period of 24 hours in rats (Roughan and Flecknell, 2000), while in mice observations of natural home cage behaviour after surgery and or anaesthesia have been recorded for periods of less than 1 to 6 hours only (Wright-Williams et al., 2007; Jacobsen et al., 2012; Jirkof et al., 2012; Leach et al., 2012; Jirkof et al., 2013a). Therefore, this study aimed to determine the effects of minor surgery (one-sided sham embryo transfer in females, one-sided sham vasectomy in males) with or without pain treatment, as well as the impact of standard, short inhalation anaesthesia alone on spontaneous and species-specific home cage behaviours in two common inbred mice strains. To this end, the overall temporal distribution of the animals' natural behaviours was investigated according to their circadian rhythmicity in order to identify whether specific behaviours are altered significantly after surgery or inhalation anaesthesia.

## 2 Methods

### 2.1 Ethics statement

The animal housing and experimental protocols were approved by the Cantonal Veterinary Department, Zurich, Switzerland, under license no. ZH 120/2008, and were in accordance with Swiss Animal Protection Law. Housing and experimental procedures also conform to European Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals used for Scientific Purposes and to the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, 2011).

### 2.2 Animals

A total of 64 C57BL/6J and DBA/2J mice of both sexes were obtained from our in-house breeding facility at the age of six to eight weeks. The health status of the animals was monitored by a health surveillance program according to FELASA guidelines throughout the experiments. The mice were free of all viral, bacterial, and parasitic pathogens listed in FELASA recommendations (Nicklas et al., 2002), except for *Helicobacter* species. All animals were housed in groups of three to eight animals of the same sex for at least three weeks prior to testing in our animal room. Animals were kept in type 3 clear-transparent plastic cages (425 mm × 266 mm × 155 mm) with autoclaved dust-free sawdust bedding and two nestlets™ (each 5 cm × 5 cm) consisting of cotton fibres (Indulab AG, Gams, Switzerland) as nesting material. Additionally, animals were provided with a transparent plastic shelter (Mouse house™, Indulab, Gams, Switzerland). They were fed a pelleted and extruded mouse diet (Kliba No. 3436, Provimi Kliba, Kaiseraugst, Switzerland) ad libitum (provided in the food hopper continuously throughout the entire duration of the experiment) and had unrestricted access to sterilised drinking water. The light/dark cycle in the room consisted of 12/12 h with artificial light (approximately 40 Lux in the cage). The temperature was  $21 \pm 1^\circ\text{C}$ , with a relative humidity of  $55 \pm 10\%$ , and the air pressure was controlled at 50 Pa with 15 complete

changes of filtered air per hour (HEPA H 14 filter). The animal room was insulated to prevent electronic and other noise. Disturbances, e.g., visitors or unrelated experimental procedures, were not allowed.

### **2.3 Experimental groups**

In order to distinguish between the effects of inhalation anaesthesia and surgery with or without analgesic treatment, 64 animals (four per sex and strain) were allocated randomly to one of four treatment groups: 1) the “anaesthesia” group (A), which received inhalation anaesthesia only; 2) the “surgery + anaesthesia + analgesia” group (S+), which underwent inhalation anaesthesia and minor surgery with analgesic treatment; 3) the “surgery + anaesthesia” group (S-), which underwent anaesthesia and minor surgery without analgesic treatment; and 4) a control group, which received no treatment (no anaesthesia, analgesia or surgery) and remained in their home cages throughout the study (C).

### **2.4 Experimental treatments and data recording**

For acclimatisation, animals were housed individually for three days as described in detail above. The experimental treatment began two hours before light phase with a subcutaneous injection of 2 µl/g body weight of phosphate buffered saline (PBS) for groups S+ and S-, and A. In the S+ group, 5 mg/kg body weight of the non-steroidal anti-inflammatory drug (NSAID) carprofen (Rimadyl™, Pfizer Inc., NY, USA) was diluted in PBS and injected as 2 µl/g body weight. Forty-five minutes later, the animals were transferred in individual transport cages to the operating theatre, which was located nearby. Mice were anaesthetised with sevoflurane (Sevorane™, Abbott, Baar, Switzerland) as a mono-anaesthesia. The anaesthetic gas was provided with a rodent inhalation anaesthesia apparatus (Provet, Lyssach, Switzerland); oxygen (100%) was used as carrier gas. After induction of anaesthesia in a Perspex induction chamber (8% sevoflurane, 600 ml/min gas flow) for two minutes, animals were transferred to a warming mat (Gaymar, TP500, Orchard Park, NY, USA) set at 39°± 1°C to ensure constant body temperature, and anaesthesia was maintained via a nose mask (4.9% sevoflurane, 600 ml/min oxygen flow). The fur was clipped and the operating field disinfected with ethanol in all



animals. Male and female mice of both surgery groups underwent a one-side sham vasectomy or a one-side sham embryo transfer, respectively. The incision in the abdominal muscle wall was closed with absorbable sutures (Vicryl™, 6/0 polyglactin 910, Ethicon Ltd, Norderstedt, Germany) and the skin was closed using skin staples (Precise™, 3M Health Care, St Paul, MN, USA). Surgery was completed within 6–8 min in both surgery groups. Anaesthesia lasted 14–16 min in all three treatment groups. Animals were allowed to recover for 15–20 min on the warming mat before being transferred back to the animal room for subsequent video recording. All experimental and control recordings began at the start of the light phase shortly after returning the mouse from its transport cage to its home cage.

## **2.5 Behavioural analysis**

Behaviour was recorded digitally in the absence of a human observer with infrared sensitive cameras (Ikegami). Each cage was recorded with one camera respectively; cameras and infrared light sources were attached 1.5 metres above the cages. The recorded material (18 hours of continuous footage) was subsequently analysed by trained and trial-blinded personnel using ObserverXT™ software (Noldus, Wageningen, Netherlands). The duration of locomotion, self-grooming, resting, eating, drinking and nest building behaviour was measured (Table 1, (Van Oortmerssen, 1970)).

Data were initially summed for the whole 18-hour period. In order to determine the temporal distribution of behavioural changes, the 18 hours were divided into three consecutive 6-hour periods according to the light-dark cycle in the animal room. Data were summed and analysed for the following time frames: 0–6 hours (light phase), 6–12 hours (light phase), and 12–18 hours (dark phase).

## **2.6 Statistical analysis**

Statistical analyses were performed with SPSS 20.0 software (IBM, Armonk, USA). All data were tested for normal distribution and homogeneity of variance. If necessary, data were log (X+1) transformed to meet the assumptions of statistical tests.

No significant effect of animal sex was detected with any of the measures. Therefore, a combined data set of males and females was used.

Mean and standard deviation (SD) of total durations of home cage behaviours were calculated.

Discriminant analysis was used to determine the effects of surgery, anaesthesia and analgesic treatment on home cage behaviour; behaviours mainly responsible for group separation were determined. The total durations of determined behaviours were further analysed using a univariate general linear model (GLM) with experimental group as a fixed factor. Post hoc tests (Bonferroni) were used for comparisons between experimental groups.

Significance for all statistical tests was established at  $p \leq 0.05$ .

### 3 Results

Contribution to group separation was analysed with discriminant analysis of the summed data, initially for the whole 18 hours observation period and subsequently for the three 6-hour periods; 0–6h; 6–12h; 12–18h (Figure 1). Behaviours that contributed most to group separations were locomotion, self-grooming and resting. These behaviours also represented the largest part of the overall behavioural time-budgets, by far. Based on this observation, only the results for locomotion, self-grooming and resting behaviour were analysed with a GLM (experimental group as fixed factor, Table 2). In case of significance post hoc tests (Bonferroni) were used for comparisons between experimental groups; these results are presented in further detail (Figure 2).

#### ***3.1 Effects of treatment on analysed behaviours: total 18-hour observations***

During the total 18-hour observation period, the control group (C) and the anaesthesia group (A) displayed significantly ( $p \leq 0.0001$ , each comparison) longer durations of locomotion compared to animals that underwent surgery with (S+) and without (S-) pain treatment (Figure 2A 1).

Self grooming behaviour was prolonged significantly after all experimental procedures compared with the untreated C group ranging from a high level in group S- ( $p \leq 0.0001$ ) to an intermediate level in group S+ ( $p \leq 0.0001$ ), with the shortest durations in group A ( $p = 0.002$ ). Additionally, significant differences between the anaesthesia and surgery groups were seen: S- ( $p \leq 0.0001$ ) and S+ ( $p = 0.004$ ) (Figure 2A 2). No significant differences in resting durations were observed between any treatments (Figure 2A 3).

### **3.2 Effects of treatment on analysed behaviours: 6-hour**

#### **observations**

By dividing the observations into 6-hour sequences, circadian differences in the effects became apparent (Figure 2B 1-3).

During the first 6h observation period (0–6h), locomotion durations did not show any significant differences between groups S-, S+, A and C. Between 6 and 12 hours post treatment, groups S-, S+ and A displayed no significant differences when compared to group C, while locomotion duration of S+ was significantly shorter than that of group A ( $p = 0.036$ ). In the last observation period (12–18h), corresponding to the first 6 hours of dark phase, all groups showed significantly shorter durations of locomotion compared to the untreated group C: S- group ( $p \leq 0.0001$ ), S+ ( $p \leq 0.0001$ ) and A ( $p = 0.006$ ). Further, duration of locomotion was significantly shorter ( $p = 0.001$ ) in group S- than in group A.

Total duration of self grooming during the first 6h observation period (0–6h) was significantly longer in experimental groups S- ( $p \leq 0.0001$ ), S+ ( $p \leq 0.0001$ ) and A ( $p \leq 0.0001$ ) compared to the control group. Additionally, there was a significant difference between groups S- and A ( $p = 0.001$ ). A comparable tendency was seen in the second time period (6–12h). Animals that underwent surgery groomed themselves for significantly longer in groups S- ( $p = 0.004$ ) and S+ ( $p = 0.042$ ), compared to group C, while in group A self-grooming was prolonged only insignificantly. In the last observation period (12–18h), animals that received surgery without

pain treatment (S-) spent the most time grooming ( $p \leq 0.0001$ ) followed by animals that received surgery with pain treatment S+ ( $p = 0.014$ ). Compared to the untreated group C, differences were significant (80 min  $\pm$  25). Animals that received anaesthesia only (A) showed significantly shorter grooming durations compared to group S- ( $p \leq 0.0001$ ). Animals that underwent anaesthesia or surgery spent significantly less time resting in the first observation period (0–6h) compared to the untreated group C: S- ( $p = 0.002$ ), S+ ( $p = 0.013$ ) and group A ( $p = 0.006$ ). In the second observation period (6–12h), resting duration was significantly shorter in group S- ( $p = 0.011$ ) compared to the untreated group C, with somewhat shorter resting duration in groups S+ and A. Animals that underwent surgery and anaesthesia spent significantly more time resting compared to untreated controls in the last observation period (12–18h): S- ( $p \leq 0.0001$ ), S+ ( $p \leq 0.0001$ ) and A ( $p = 0.045$ ).

#### 4 Discussion

To assess the impact of inhalation anaesthesia and surgery with or without pain treatment in mice, we used non-invasive behavioural recordings that can be applied in the animals' home cage without disturbing the animal or provoking additional stress. Using this system we were able to analyse each animal's behaviour continuously for 18 hours following experimental treatments. The results of our study showed that minor surgery with short inhalation anaesthesia, either with or without pain treatment, induced alterations in spontaneous home-cage behaviours such as self-grooming, resting and locomotion. These changes persisted for up to 18 hours.

When interpreting the data summed over the whole 18-hour observation period, we found that locomotion and self-grooming behaviours were most affected by the experimental procedures. After surgery, animals displayed a marked decrease in locomotion and a strong increase in self-grooming. Self-grooming showed a clear stepwise increase from baseline over anaesthesia only, to surgery with pain treatment, to surgery without pain treatment. In contrast to locomotion and self-grooming, there was no effect on the overall duration of resting behaviour if the 18-hour post treatment period was observed as a whole.

Pain treatment with carprofen had no significant effect on spontaneous home cage behaviours. However, animals that received pain treatment during surgery readily reached intermediate levels of locomotion and self-grooming compared to the group in which pain treatment was not administered during surgery. Therefore, it could be speculated that some, but not complete, amelioration of post-operative pain is achieved by administering the non-steroidal anti-inflammatory drug (NSAID) carprofen at a dosage of 5 mg/kg body weight. However, previous studies using physiological investigations and behavioural testing demonstrated that carprofen provided sufficient relief from post-operative pain (Arras et al., 2007; Jirkof et al., 2010). Thus, it might be that the alterations of spontaneous home cage behaviours analysed in this study are not ideal parameters for estimating pain alleviation by NSAIDs.

When dividing the observation period into three consecutive 6-hour-long time segments according to the light cycle in the animal room, circadian-dependent display patterns could be observed. For the first 12 hours after treatment—corresponding to the complete light phase in the animal room—all animals displayed general low levels of locomotor activity, as expected for mice (Kramer et al., 2001). No difference could be observed between treated and untreated groups regarding this behaviour. However, in post-treatment hours 12–18 (first 6 hours of the dark phase) locomotion of both surgery groups was reduced by 75% and that of the anaesthesia group by 35% compared to that of the control group. Reduced activity after surgery has been observed in mice for example also after hepatectomy as well as telemetry transponder implantation (Blaha and Leon, 2008; Tubbs et al., 2011).

In contrast, the duration of self-grooming behaviour was influenced strongly by treatment in all three time segments, showing a marked increase in treated animals as compared to the untreated control group. Even though, self-grooming behaviour is known to have a circadian rhythm with a frequency peak shortly before dark phase in healthy mice (Poirel, 1988), treatment effects on duration of self grooming seemed not to be influenced by the light-dark cycle in the animal room in our study. Self-grooming can be displayed as displacement or adjunctive behaviour in rodents (Spruijt et al., 1992). Stressors like novelty and handling can elicit grooming behaviour in rats (Bindra and Spinner, 1958; Colbern et al., 1978) and

increased grooming is known to occur after surgery in mice (Van Loo et al., 2007; Jirkof et al., 2012; Leach et al., 2012; Jirkof et al., 2013a). While no correlation with indicators of anxiety were found, it seems that grooming coincides better with the period after arousal and rather reflects the process of habituation to a stressful situation (Spruijt et al., 1992). Moreover, central dopaminergic activation, most likely via the D1 receptor, was reported to induce intense grooming behaviour (Toyoshi et al., 1992; Stoessl, 1994; Marin et al., 1996; Imaizumi et al., 2000). Interestingly, it has been shown that a D1 receptor-mediated arousal mechanism likely plays also an important role in emergence from general anaesthesia (Taylor et al., 2013).

The duration of resting behaviour, which displayed no differences between groups in the summed 18-hour analysis period, showed clear and gradual treatment-related effects when analysed according to the time progression, i.e. in separate 6-hour periods. Effects were most significant during the first 6 hours after treatment, when resting decreased, and at 12–18 hours after treatment, when it increased markedly in comparison to control animals. Healthy mice mostly rest during the light phase and show a stable circadian rhythm; disruption of this rhythm as observed in this study might indicate impaired wellbeing (Landis et al., 1988; Jirkof et al., 2012; Jirkof et al., 2013a).

In recent years, volatile anaesthetics (e.g., sevoflurane, isoflurane) have been used increasingly in laboratory animal practice due to their safety and association with rapid recovery. Further, inhalation anaesthesia is used in diverse procedures, including research in which animals are studied subsequently in behavioural tests and where their performance may be influenced by the persistent effects of anaesthetic drugs (Valentim et al., 2008). Previous studies have demonstrated that inhalation anaesthesia can induce changes in heart rate, core body temperature and faecal corticosterone levels that last for several hours after treatment (Wright-Williams et al., 2007; Cesarovic et al., 2010). In this study, we have shown that non-painful, short (15 minutes) sevoflurane inhalation anaesthesia can cause post-anaesthetic alterations in locomotion, self-grooming and resting behaviours that are noticeable for up to 18h after treatment. Therefore, for accurate interpretation of behavioural research data the distinct individual impacts of anaesthesia, surgery, pain treatment and other experimental

procedures have to be considered. However, as our study protocol did not determine the extent of effects caused by treatment-related actions (like transport, handling etc.), this question requires further investigation.

Studying behaviour in sufficient detail to detect post-surgery-related changes in spontaneous, home-cage based behaviour patterns, and the effects of drugs upon such behaviours, is quite tedious and time consuming, thus studies are often confined to analysing only a limited range of behaviours or performing assessments only over a very short time frame (Roughan et al., 2009). Determining an optimal observation time-point is one of the major difficulties in behavioural research. For example, the display of signs of pain as well as pain tolerance itself is dependent on circadian rhythm, and thus the need to determine the appropriate time of day for observations (Chassard and Bruguerolle, 2004; Oishi et al., 2007; Matsumiya et al., 2012) renders post-operative pain assessment even more challenging in mice. In this study, we were able to show that all 3 behaviours studied in detail (locomotion, self-grooming and resting) displayed different temporal effect patterns. Whereas the effects on self-grooming were distributed evenly over the whole period analysed, locomotion was changed only during 'dark' and resting first decreased and then increased markedly in treated groups. We believe that these data suggest strongly that the effects on spontaneous, home-cage based behaviours caused by anaesthesia and minor surgery are not displayed uniformly throughout the day. However due to the experimental design it is not possible to determine to which extend the differences observed between the observation phases were linked to circadian effects or time since experimental manipulation or if interactions between these factors occurred. Nevertheless, our results highlight the requirement for knowledge of species-specific circadian rhythms of behaviours as well as the importance of determining the appropriate time of day for behavioural and welfare assessment.

## 5 Conclusion

Spontaneous home cage behaviours, locomotion, self-grooming and resting, differed between untreated control, anesthesia and surgery groups for up to 18 hours post treatment. Short

inhalation anesthesia induced moderate changes whereas the impact of surgery was considerable. Thus it can be assumed that the observed changes in home cage behaviors hint at reduced animal well-being. Pain therapy only partially ameliorated the aforementioned effects, leading to the conclusion that either the chosen dosage was too low or that alterations in the spontaneous home cage behaviors analyzed in this study do not allow NSAID efficiency to be estimated reliably.

While self grooming behavior changed post experimentally independently of circadian rhythm, changes in locomotion and resting behavior were distinctly affected by the time of day.

In conclusion, for proper interpretation of behavioural research data, the distinct impacts of anaesthesia, surgery, pain treatment and other experimental procedures have to be considered. Our results highlight the requirement for knowledge of species-specific circadian rhythms of behaviours as well as the importance of determining the appropriate time of day for behavioural and welfare assessment.

## 6 Acknowledgements

This work was sponsored by grants from the Federal Veterinary Office (Bern, Switzerland), and UBS foundations. The authors would like to thank Robin Schneider, Hugo Battaglia and the staff of the Central Biological Laboratory for support in housing mice. We thank Professor Kurt Bürki for generously providing research facilities and resources.

## 7 Disclosure Statement

The authors have no conflict of interest to disclose.



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## Table and figure captions

**Table 1:** Ethogram of home cage behaviours according to Van Oortmerssen (1970).

**Table 2:** Results of GLM analyses with experimental group as fixed factor for resting, locomotion and self-grooming duration.

**Figure 1:** Scatter plot of discriminant scores assigned to individual mice. The significance of each function in separating groups, and their percentage contribution to between-group variance are shown on each axis. **A)** 18h observation period. Duration of self grooming and locomotion behaviour contributed most to group separation. **B)** During the first observation sequence (0-6h post treatment) locomotion and self-grooming behaviour were mainly responsible for group separation. **C)** During 6–12h post treatment, duration of self-grooming behaviour was mainly responsible for group separation. **D)** Locomotion, self-grooming and resting contributed significantly to group separation during the 12–18h post-treatment observation period.

**Figure 2:** Effects of anaesthesia and surgery with or without analgesic treatment on duration of 3 spontaneous home-cage behaviours compared to control values. \* Significant ( $p \leq 0.05$ ) differences between experimental groups. **A)** Total duration of locomotion (A1) was decreased post experiment, self-grooming (A2) increased, and resting (A3) behaviour remained unchanged when the whole 18h observation period was analysed. **B)** Temporal distribution of behavioural effects became apparent when dividing observations into 3 consecutive 6-hour sequences. Duration of locomotion behaviour (B1) was unchanged during the lights-on period but shortened dramatically during the dark period. The increase in duration of self-grooming

541 (B2) was distributed equally in all time sequences, whereas resting (B3) was decreased during  
542 the first (0–6h) and increased during the last (12–18h) sequence.  
543

543

| home cage behaviours |   |
|----------------------|---|
| resting              | motionless state, no activity (sitting or lying flat, sometimes with the eyes closed or nearly closed, includes sleeping) |
| locomotion           | oriented movement including walking, running, jumping and climbing at the cage grid                                       |
| self grooming        | wiping, licking and nibbling the fur with forepaws and tongue, but also scratching and claw cleaning                      |
| eating               | consumption of food   |
| drinking             | consumption of water from the water bottle  |
| nest building        | carrying and shredding of the nestlet, arrangement of cotton fibres, creation of a nest                                   |

544 **Table 1: Ethogram of home cage behaviours** according to Van Oortmerssen (1970).

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546 **Table 2: Results of GLM analyses with experimental group as fixed factor for resting,**

| behaviour     | observation duration        |                                | locomoti<br>on and<br>self-<br>groomin<br>g<br>duration |
|---------------|-----------------------------|--------------------------------|---|
|               | 18h: F = 2.015; p = 0.121   | 0-6h: F = 6.202; p = 0.001     |   |
| resting       |                             | 6-12h: F = 3.652; p = 0.017    |   |
|               |                             | 12-18h: F = 9.984; p ≤ 0.0001  |   |
| locomotion    | 18h: F = 23.658; p ≤ 0.0001 | 0-6h: F = 3.344; p = 0.025     |   |
|               |                             | 6-12h: F = 2.760; p = 0.050    |   |
|               |                             | 12-18h: F = 21.826; p ≤ 0.0001 |   |
| self grooming | 18h: F = 38.162; p ≤ 0.0001 | 0-6h: F = 27.845; p ≤ 0.0001   |   |
|               |                             | 6-12h: F = 5.105; p = 0.032    |   |
|               |                             | 12-18h: F = 11.273; p ≤ 0.0001 |   |

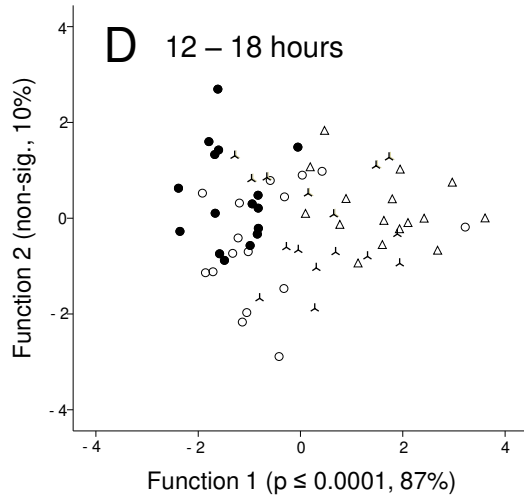
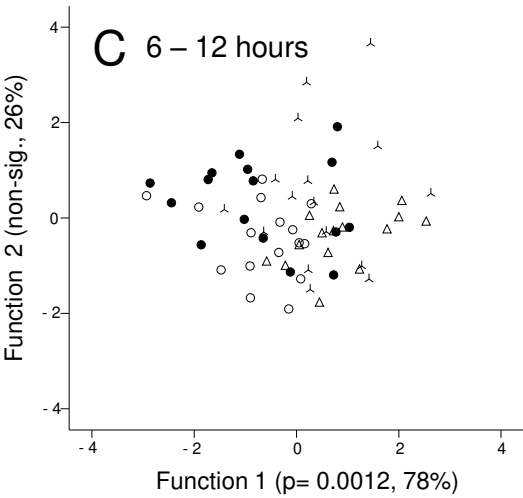
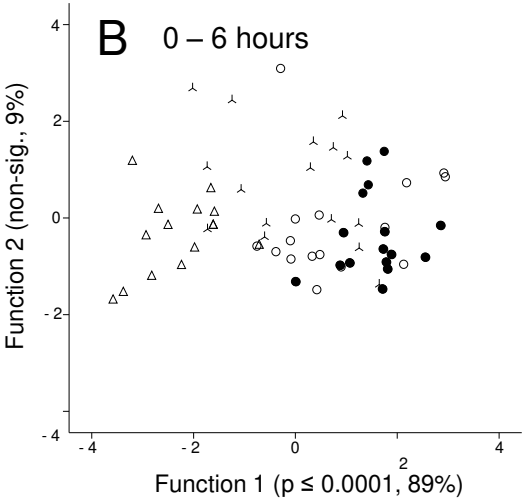
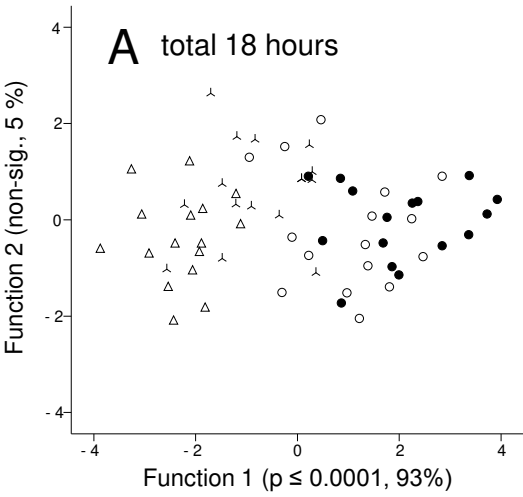
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554 Highlights  
555 Effects of anaesthesia on mouse behaviour are analysed rarely  
556 Here we report clear post-anaesthetic and surgical changes in home cage behaviours  
557 Resting and locomotion altered contradictory to their circadian rhythm  
558 Self-grooming was increased independently of circadian rhythm  
559 Knowledge of circadian rhythms is essential for behavioural and welfare assessment  
560



Figure 1



- Surgery + anaesthesia
- Surgery + anaesthesia + analgesia
- ▲ Anaesthesia
- △ Control

Figure 2

